CTP can replace GTP in reactions catalyzed by eukaryotic peptide elongation factor 1

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In several reactions catalyzed by highly purified peptide elongation factor 1 from rabbit reticulocytes, GTP may be fully replaced by CTP but not by ATP or UTP. This holds true for the factor-dependent binding of aminoacyl-tRNA to ribosomes, GTPase activity, GTP-dependent autophosphorylation of the factor protein and binding of cholesteryl 14-methylhexadecanoate by the factor.

CTP *GTP* *Peptide elongation factor 1* *GTPase* *Autocatalytic phosphorylation* *Cholesteryl 14-methylhexadecanoate*

1. INTRODUCTION

Guanosine triphosphate is a cofactor absolutely required for several steps involved in gene translation. Its presence is necessary for binding of aminoacyl-tRNA to ribosomes catalyzed by eEF-1 [1] which represents the initial stage of peptide elongation. This protein-synthesis factor forms a binary complex with GTP [2] and combines also with aminoacyl-tRNA and GTP to form a ternary complex which is an essential intermediate in the binding of aminoacyl-tRNA to ribosomes [3]. Peptide-elongation factor exhibits a ribosome-independent GTPase activity [4,5] and, similarly as eIF-2 [6], a GTP-dependent autophosphorylation which is apparently an important regulatory mechanism in the function of this protein-synthesis factor [5].

The results presented here provide evidence that in reactions catalyzed by eEF-1, GTP can be replaced by CTP but not by other nucleotides (ATP and UTP).

Abbreviations: eEF-1, eukaryotic peptide elongation factor 1; eIF-2, eukaryotic peptide initiation factor 2

2. MATERIALS AND METHODS

Sodium salts of GTP, ATP and UTP were purchased from Koch-Light, Colnbrook, England, and the sodium salt of CTP originated from Sigma, St. Louis, MO. [γ-^{32}P]GTP (20 Ci/mmol) was a product of Amersham, England and [γ-^{32}P]CTP (1.1 Ci/mmol) was synthesized by a modified mixed anhydride technique [7]. Briefly, CDP (Reanal, Budapest) was reacted with ^{32}P and ethylchloroformiate in dimethylformamide in the presence of triethylamine at -20°C. Dimethylformamide was removed by azeotropic distillation with acetonitrile and the product was separated by chromatography on polyethylene imine-cellulose. Both radioactive nucleotides were diluted with corresponding non-labeled compounds if required.

Peptide elongation factor 1 was isolated from rabbit reticulocytes as described [8] and purified to electrophoretic homogeneity [5]. The poly(U)-dependent binding of [^{3}H]Phe-tRNA catalyzed by eEF-1 was assayed as described [5]. Assays for the GTPase activity and for the autophosphorylation of eEF-1 were performed as described for eIF-2 [6].

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3. RESULTS AND DISCUSSION

The poly(U)-dependent binding of [^{3}H]Phe-tRNA to ribosomes was, as expected, significantly enhanced in the presence of GTP, the optimum concentrations of this nucleotide being in the range of 0.3–0.5 mM. The relatively high background value for the binding obtained in the absence of GTP was obviously due to the presence of endogenous GTP in eEF-1 preparations. Even homogenous preparations of eEF-1 contain 0.21–0.43 pmol of GTP/pmol of eEF-1, as demonstrated earlier [5].

A similar stimulating effect on the binding reaction with GTP was also found with CTP. The overall effect was essentially the same, but higher concentrations (optimum approx. 1 mM) of CTP were required when compared with GTP. If a combination of GTP and CTP was added into mixtures, the stimulating effect was higher than if GTP or CTP were used alone (fig.1). Both ATP and UTP were without effect on the binding of [^{3}H]Phe-tRNA to ribosomes.

Peptide elongation factor 1 was demonstrated to exhibit a ribosome-independent GTPase activity [4,5]. However, the factor was able to hydrolyze not only GTP but also CTP. The efficiency of the splitting of this latter nucleotide was comparable with that obtained with GTP (fig.2). However, under the same conditions the factor did not show any ATPase or UTPase activity.

The protein-synthesis factor used is capable of a GTP-dependent autophosphorylation, similar to eIF-2 [6], and evidence has been presented that this reaction represents a regulatory mechanism in eEF-1 activity [5]. Not only GTP but also CTP may be utilized as a substrate for this autophosphorylation with an efficiency comparable to GTP (fig.3). ATP and UTP are unable to phosphorylate eEF-1 protein, apparently because both these nucleotides are not hydrolyzed by eEF-1 [5].

The binding of cholesteryl 14-methylhexadecanoate by eEF-1 [9] is also significantly stimulated, not only by GTP but also by CTP [5], whereas both ATP and UTP are ineffective in this respect.

It is not clear at the moment why only CTP, and not the other two nucleotides, is able to replace fully GTP in reactions catalyzed by eEF-1. Both GTP and CTP have a quite dissimilar composition, one of them containing a purine and the other a pyrimidine base. However, both these bases are complementary and it has been postulated [10] that such complementary bases may become bound with similar interaction energies to the same amino

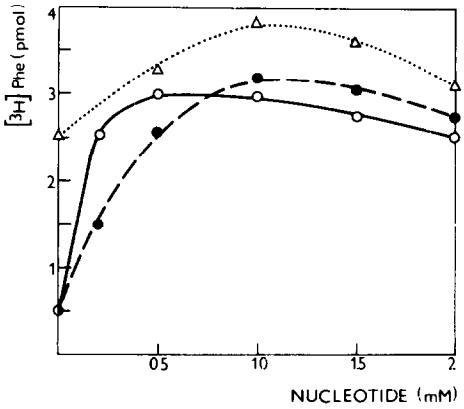

Fig.1. The binding of [^{3}H]Phe-tRNA to ribosomes in the presence of GTP (○), CTP (●) and a combination of GTP and CTP (△). Incubation mixtures composed as described [5] contained 20 μg eEF-1, quantities of [^{3}H]Phe-tRNA corresponding to 6 pmol of phenylalanine, concentrations of GTP or CTP alone as indicated or 0.2 mM GTP together with CTP as indicated, and were incubated at 37°C for 30 min.

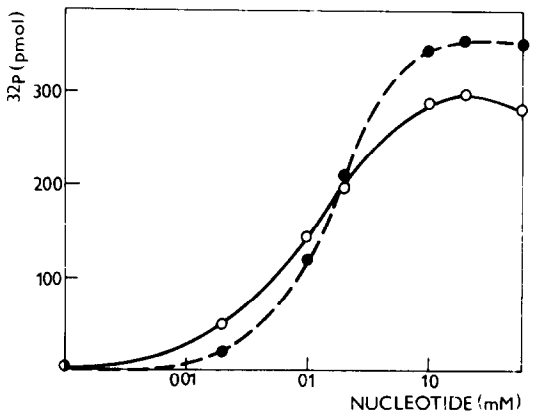

Fig.2. Liberation of inorganic phosphate from [γ-^{32}P]GTP (○) and from [γ-^{32}P]CTP (●) in the presence of eEF-1. Incubation mixtures composed as described [6] contained 15 μg eEF-1, concentrations of GTP or CTP as indicated and were incubated at 37°C for 40 min.

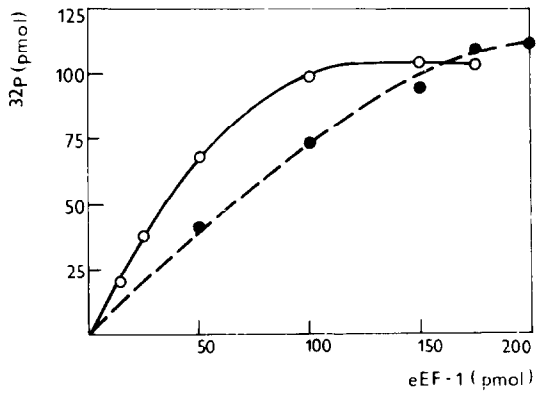

Fig.3. Autophosphorylation of eEF-1 protein from GTP (○) or CTP (●). Incubation mixtures composed as described [6] contained 1 mM GTP or 1 mM CTP, quantities of eEF-1 as indicated, and were incubated at 37°C for 40 min.

acid residues in the binding site of a given enzyme or protein factor.

CTP has been found to be an essential cofactor involved in the incorporation of phosphoryl choline into lipids [11] and required for the synthesis of CDP-choline and CDP-ethanolamine [12,13]. It also acts as a specific allosteric inhibitor of aspartate transcarbamylase in *Escherichia coli* [14,15]. However, the involvement of this nucleotide in protein synthesis has not yet been reported.

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